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R C van Dijk

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Anmelder/Applicant(s)/Demandeur(s):

Technische Universität München
Arcisstrasse 21
80333 München
ALLEMAGNE
Tularik Inc.
1120 Veterans Boulevard
South San Francisco, CA 94080
ETATS-UNIS D'AMERIQUE

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TLR2 antagonistic antibody and use thereof

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TLR2 antagonistic antibody and use thereof

Introduction

Host cells recognize specific patterns of microbial components through Toll-like receptors (TLRs) which are crucial in mediating innate immune responses[1,2]. Lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria is a potent agonist for TLR4 whose effects on the host organism have been studied extensively in experimental models of infection and septic shock[3-5]. Over-stimulation of host immune cells by microbial products accompanied by the release of large amounts of inflammatory mediators is recognized as a major cause of septic shock[6-9].

Indeed, this concept has been validated by using both gene targeted mice lacking the expression of the respective receptors, and by receptor specific inhibition of microbial product induced host cell activation. For example, the non-redundant role of CD14 as an important element of a cellular LPS recognition system has been demonstrated by application of inhibitory anti CD14 antibodies in rabbits[10,11]. The blockade of LPS receptors or extracellular effector proteins as the earliest possible targets of therapeutic strategies was shown to be preventive[12]. Another approach of therapeutic intervention in septic shock has been interference with the functions of proinflammatory cytokines such as TNFα or IL-1β. For instance, competitive inhibition of cytokine binding to its signaling receptors by application of recombinant extracellular domain (ECD) or naturalizing receptor antagonist proteins have been shown to be protective in LPS induced shock in rats[13]. In addition, antagonistic antibodies targeting cytokines or ECDs of its receptors have been tested[14]. While cytokine blockade for therapeutic intervention in acute infections (sepsis) has been disappointing[15], its use in treatment of chronic inflammations is promising[16,17].

Besides Gram-negative, Gram-positive bacteria lacking LPS play an important role in the clinical manifestation of shock[8]. Cell wall components from these bacteria such as peptidoglycan (PGN) and lipoteichoic acid (LTA) are considered the main causative agents of Gram-positive shock[18,19]. PGN is a main component of Gram-positive, but also of other bacterial cell walls, and consists of an alternating β (1,4) linked N-acetylmuramyl and N-acetylglucosaminyl glycan cross linked by small peptides[20]. In contrast, the macroamphiphile LTA, a saccharide chain molecule consisting of repetitive oligosaccharides connected by alcohols such as ribitol and carrying acyl chains through which it is anchored to the bacterial cytoplasmic membrane, is specific for Gram-positive bacteria[21]. For example, LTA has been described to carry the major stimulatory activity of *Bacillus subtilis*[22]. The stimulatory properties of tripalmityolated proteins whose production is not restricted to Gram-negative or Gram-positive bacteria are mimicked by the synthetic compound N-palmitoyl-S-(2,3-bis(palmitoyloxy)-(2R,S)-propyl)-(R)-cysteinyl-seryl-(lysyl)3-lysine (P_3CSK_4)[23].

Most of these bacterial products are known to activate innate immune cell responses by triggering the TLR2 signaling cascade[2]. The TLR2ECD, whose N-terminal portion has been implicated in PGN recognition[24], contains an array of distinct leucine rich repeat (LRR) motifs[25]. The LRR rich domain is followed by an LRR C-terminal (LRRCT), a trans-membrane, and an intracellular C-terminal Toll – IL-1 receptor – plant disease resistance protein (TIR) domain[25].

Here, we show that a murine monoclonal antibody (mAb) raised against the murine TLR2ECD inhibits TLR2 mediated activation of murine and human cells. Using two TLR2 dependent shock models, we demonstrate the protective potential of neutralizing TLR2 with this antibody *in vivo*. We propose that antibody targeting of the TLR2ECD is a valuable strategy to prevent TLR2 driven septic shock.

Abbreviations used: TLR, Toll-like receptor; mAb, monoclonal antibody; P_3CSK_4, tripalmitoyl-cysteinyl-seryl-(lysyl)3- lysine; h. i. *B. subtilis*, heat inactivated *Bacillus subtilis*; sPGN, soluble peptidoglycan; LTA, lipoteichoic acid

Results

Application of murine mAb T2.5 for expression analysis *in vitro*

We have selected an IgG1κ anti TLR2 mAb named T2.5 which specifically recognized TLR2. HEK293 cells stably expressing murine or human TLR2 were stained specifically on their surface by T2.5 (Fig. 1*a* and *b*). Furthermore, T2.5 did not bind to primary murine *TLR2*$^{-/-}$, but bound to wild-type macrophages cultured *in vitro* (Fig. 1*c* and *d*). T2.5 immuno-precipitated native murine and human TLR2 from lysates of HEK293 cells overexpressing each of the two TLR2 orthologs (Fig. 1*e*). Most importantly, T2.5 precipitated endogenous TLR2 from lysates of RAW264.7 macrophages (Fig. 1*e*). Next, we analyzed T2.5 for its capacity to specifically detect TLR2 on the subcellular level. Overexpressed murine and human TLR2 (Fig. 2*a*), as well as endogenous TLR2 were detectable in primary murine and CD14^{+} leukocyte derived human macrophages (Fig. 2*b*).

Inhibitory effects of T2.5 on TLR2 specific cell activation

T2.5 inhibited murine and human TLR2 mediated cell activation by TLR2 specific stimuli P_3CSK_4 or *B. subtilis* applied to HEK293 cells overexpressing TLR2, murine RAW264.7, and primary macrophages as measured by NF-κB dependent reporter gene assay and IL-8 specific enzyme linked immuno sorbent assay (ELISA), as well as TNFα and IL-6 specific ELISA, respectively (Fig. 3*a* to *d* and data not shown). A second newly generated IgG1κ anti TLR2 mAb, conT2, was used as a control. This mAb binds native murine (m) TLR2 in a manner comparable to T2.5 but not human TLR2 (data not shown) and failed to inhibit TLR2 dependent cell activation (Fig. 3). Also, no inhibition of Il-1 receptor or TLR4 signaling by T2.5 was detected, indicating that TLR2 independent signaling pathways in T2.5 treated cells remain intact (Fig. 3*a* to *d*). Moreover, TLR2 mediated nuclear translocation of NF-κB was specifically inhibited by T2.5 in human macrophages (Fig. 3*e*). NF-κB specific electro mobility shift assay (EMSA), as well as anti phospho p38, Erk1/2, and Akt immunoblot analysis revealed T2.5 but not conT2 dose dependent inhibition of P_3CSK_4 induced NF-κB - DNA binding and cellular kinase phosphorylation (Fig. 3*f* and *g*).

Flow-cytometry of intracellular and surface TLR2 expression ex vivo

Since LPS induces TLR2 expression in primary macrophages *in vitro* (data not shown), we first compared T2.5 specific staining of $CD11b^+$ splenocytes from LPS challenged wild-type and *TLR2*$^{-/-}$ mice by flow-cytometry. Weak surface staining and pronounced intracellular staining were seen (Fig. 4*a*). In subsequent experiments, peritoneal cells and splenocytes from mice infected with the Gram-positive bacterium *B. subtilis* were analyzed. While surface expression of TLR2 in primary murine macrophages was relatively strong upon *in vitro* culture (Fig. 1*d*), surface expression was weak or not detectable in unchallenged $CD11b^+$, $CD11c^+$, $CD19^+$, and $GR1^+$ subpopulations of splenocytes and peritoneal washout cells (Fig. 4*a*, *b* and data not shown). Upon microbial challenge, however, TLR2 surface expression strongly increased in $CD11b^+$ and $GR1^+$ cells (Fig. 4*b* and data not shown). Signals were specific as tested by analysis of *TLR2*$^{-/-}$ cells (Fig. 4). Again, intracellular staining of TLR2 revealed significant levels of intracellular TLR2 expression which increased to a higher degree than surface expression upon microbial challenge (Fig. 4*a* and *b*).

Antibody mediated interference with TLR2 specific immune responses towards systemic challenge

Next, we determined cytokine and chemokine serum concentrations in mice, either pretreated, or not pretreated with T2.5, as well as challenged with P_3CSK_4. While cytokine and chemokine concentrations were low in sera of untreated mice (see methods), serum levels of TNFα, IL-8, IL-6, and IL-12p40 were significantly lower in mice preinjected with T2.5 as compared to controls and measured after challenge (Fig. 5*a* to *d*).

Both, a high dose (microbial product only) and a low dose model (additional sensitization with D-galactosamine) have been established for bacterial product induced shock in mice[26]. In order to interfere in a strictly defined model of septic shock, we applied the bacterial lipopeptide analogue and TLR2 agonist P_3CSK_4 upon sensitization of mice with interferon gamma (IFNγ) and D-galactosamine[27]. Sensitization was used to mimic priming of a host defense towards further microbial

challenges by an underlying infection. While mice that had received no mAb or conT2 30 min prior to injection succumbed to lethal shock within 24 h, mice treated with T2.5 survived (Fig. 6*a*). Intending to employ a complex challenge mimicking infection for a distinct shock model, we took advantage of the finding that shock induction by viable or heat inactivated Gram-positive *B. subtilis* bacteria is TLR2 dependent not only in a low dose, but also in a high dose model (unpublished observation). Mice were pretreated with T2.5 or conT2 followed by challenge with a lethal dose of *B. subtilis* (protective protocol). In a separate group of mice, we first administered *B. subtilis* and applied T2.5 up to 3 h later (therapeutic protocol). In the absence of T2.5 the high dose *B. subtilis* challenge was lethal for all mice tested (Fig. 6*b*). However, when given T2.5 either prior (1 h), or up to 2 h after microbial challenge, all *B. subtilis* challenged mice survived. Most notably, treatment with T2.5 even 3 h after potentially lethal injection saved 75% of mice challenged (Fig. 6*b*).

Discussion

Our results show a therapeutically useful function of an antagonistic TLR2 mAb in TLR2 driven septic shock. We found that application of TLR2 agonists was lethal in two experimental models of septic shock and therefore aimed at identification of antibodies blocking TLR2. Here we show that the mAb T2.5 prevents P_3CSK_4, a synthetic analogue of bacterial lipopeptides, or Gram-positive bacteria (*B. subtilis*) induced shock in mice. T2.5 also blocks human TLR2 function, since subcellular NF-κB translocation upon TLR2 specific challenge of primary human macrophages was inhibited upon its application.

The lack of TLR functions negatively affects humans at least upon acute infections[28,29]. In a systemic model of polymicrobial sepsis encompassing standardized influx of the gut flora into the peritoneal cavity, however, mice benefit from the lack of TLR functions[30] indicating TLR dependent mediation of harmful effects in acute infection. Indeed, blockade or application of LPS binding proteins such as CD14, bactericidal/ permeability-increasing protein (BPI), or LPS binding protein (LBP) has been effective to inhibit LPS induced pathology[31-34] even after LPS application. Since this exemplified the prevalent role of a single cellular system for specific recognition of a microbial product[35,36], we attempted to intervene in cell activation by TLR2 specific microbial products. The finding that T2.5 recognized murine and human TLR2 on the cell surface of and within cells cultured *in vitro*, as well as exhibited antagonistic effects upon application to cells expressing these receptors provided a basis for our attempt. Furthermore, the antagonistic function was specific and dose dependent.

We analyzed the potential of T2.5 to prevent TLR2 driven immunopathology. Application of T2.5 30 min or 1 h prior to application of lethal doses of TLR2 specific agonists P_3CSK_4 to sensitized mice or of *B. subtilis* to normal mice, respectively, protected mice against their lethal effects (Fig. 6*a* and *b*), but not against the lethal effects of LPS (data not shown). In fact, *B. subtilis* induced shock was prevented upon application of T2.5 2 h, or even 3 h after shock induction (100% or 75% of survival, respectively). Our results indicate complement mediated depletion of $TLR2^+$ cells as an unlikely

mechanism of T2.5 mediated shock prevention, since application of the mTLR2 specific isotype matched mAb conT2 *in vivo* did not result in protection. This implicates reversibility of mAb mediated TLR2 blockade which is potentially important for timely recovery of TLR2 dependent cellular responsiveness in later phases of sepsis at which diminished immune function is fatal[9]. The demonstration of beneficial effects of T2.5 in both, a sensitization dependent and a high dose TLR2 specific experimental model, validate a therapeutical application in the face of many animal models being restricted to one of the two respective dosages. This characteristic may improve transferability of the results from our animal study to treatment of sepsis in humans[9].

Perhaps it is the surprisingly very low constitutive surface expression of TLR2 in host cells such as $CD11b^+$ (macrophage), $GR1^+$ (granulocyte), $CD19^+$ (B cell), $CD11c^+$ (dendritic cell) splenocytes, and peritoneal cells *in vivo* (Fig. 4 and data not shown), which explains the efficacy of T2.5 mediated prevention of shock triggered via TLR2. This low surface expression is in contrast with relatively high surface expression in unchallenged primary murine (Fig. 1), as well as human myeloid cells upon *in vitro* culture[37] giving account for immediate TLR2 expression analysis *ex vivo*. However, comparison of TLR2-staining of non-permeabilized and permeabilized cells indicates that a major portion of TLR2 was localized in the intracellular compartment of murine $CD11b^+$ and $GR1^+$ cells, as well as human macrophages (Fig. 4*c*, unpublished observation and Fig. 2*b*). In fact, we noted increased surface and intracellular TLR2 expression in specific cell populations 24 h after bacterial infection which was similar upon LPS challenge (Fig. 4 and data not shown). The time course of TLR2 regulation in distinct immune cells upon microbial contact needs to be investigated in more detail because it might determine the time frame in which TLR blockade based intervention strategies can be effective.

Antagonistic properties have recently been demonstrated *in vitro* also for two anti human TLR2 mAbs[38,39] possibly indicating distinct active complex formation of TLRs as compared to receptors for which agonistic antibodies have been identified. However, T2.5 interferes with the lipopeptide/TLR2

complex that induces cell activation, as well as recognizes a human TLR2 construct lacking the N-terminal third of the TLR2 LRR rich domain (data not shown) we found to be dispensable for cellular recognition of lipopeptides[40]. Thus, the epitope recognized by T2.5 must be located within the C-terminal portion of the TLR2ECD. We expect that identification of the epitope will show its conservation between mice and humans. The potential of T2.5, for instance in combination with further inhibitors of inflammatory processes, to inhibit pathogenesis of clinically important infections awaits its evaluation. In conclusion, our data are the first to point out the potential of TLR2 specific antibody application as a therapeutic strategy to block TLR2 mediated cell activation in the course of acute infection through *in vivo* evidence.

Methods

Material. Over night (o. n.) *B. subtilis* (DSMZ.1087) cultures containing approximately 1×10^{10} colony forming units (cfu)/ml (brain heart medium) were used immediately or heat inactivated (h. i.) at 56°C for 50 min. Synthetic P_3CSK_4 was purchased from ECHAZ microcollections (Tuebingen, Germany), ultra pure LPS from *Salmonella minnesota Re595* was from List Laboratory (Campbell, California), recombinant murine IFNγ and IL-1β from Peprotech (London, England), and D-galactosamine from Sigma (Deisenhofen, Germany).

Mice. Matched groups of wild-type ($TLR2^{+/+}$) B57BL/6 and $TLR2^{-/-}$[27] mice kindly provided by Tularik (generated by Deltagen; South San Francisco, California; nine-fold crossed towards B57BL/6 background) were applied.

Generation of TLR2ECD specific antibodies and ELISA. A cDNA fragment encoding the N-terminal 587 amino acids of mTLR2[41] was amplified from a RAW264.7 cDNA library (advantage kit, BD Clontech, Heidelberg, Germany). The murine TLR2ECD was fused to a C-terminal thrombin cleavage site followed by a human IgGFcγ moiety. The murine TLR2ECD protein was purified upon overexpression in HEK293 cells and thrombin digestion. A $TLR2^{-/-}$ mouse was immunized by intraperitoneal (i. p.) injection of 50 μg of TLR2ECD and 10 nmol of a thioated DNA oligonucleotide (5'-TCCATGACGTTCCTGA-3', Tib Molbiol, Berlin, Germany) for three times within eight weeks. Its splenocytes were fused with murine P3X cells and hybridomas were selected[42]. MAb specificities for TLR2ECD, as well as cyto- and chemokine concentrations in cell supernatants or murine sera (see below) were analyzed by ELISA (R&D systems, Minneapolis, Minnesota).

Flow cytometry. Stably transfected HEK293 cell clones, as well as uninduced peritoneal wash-out macrophages were cultured o. n. as described[40]. Flow cytometry was performed upon staining with either T2.5, or affinity purified polyclonal rabbit antisera specific for the murine TLR2ECD[43] or the Flag tag (Sigma), as well as respective secondary mAbs (BD Pharmingen, Heidelberg, Germany).

For establishment of mTLR2 expression analysis in primary cells, surface and intracellular T2.5 dependent staining of $CD11b^+$ splenocytes[42] from wild-type and *TLR2*$^{-/-}$ mice challenged with LPS (0.5 mg, i. p., 24 h) were compared by flow cytometry (CyAn, Dako Cytomation, Fort Collins, Colorado). Cells were stained with photoactivated ethidium monoazide (Molecular Probes, Amsterdam, Netherlands) immediately upon isolation, followed by TLR2 specific surface staining, or intracellular staining (cytofix/cytoperm, BD Pharmingen). In order to analyze TLR2 expression in non- or *B. subtilis* infected (5×10^{10} cfu, i. p., 24 h) mice, peritoneal washout cells and splenocytes[42] from five wild-type or *TLR2*$^{-/-}$ mice were pooled, respectively. Fluorescence labeled cell surface marker antibodies (BD Pharmingen) and primary T2.5 stained with secondary anti mIgG1 were used as indicated.

Immunoprecipitation and immunoblot analysis. Lysates of Flag-TLR2 transfected HEK293 cells or macrophages, as well as 1 μg of antibody and protein G beads (Santa Cruz, California) were mixed for o. n. precipitation. Immune complexes or cell lysates were analyzed by immunoblot analysis as described[40]. Precipitations were controlled by application of Flag specific (mAb M2, Sigma) or protein G beads only. Flag (HEK293) or mTLR2[43] (RAW264.7) specific antisera were applied for immunoblot analyses. In contrast, total lysates of macrophages (see inhibition experiments) were analyzed for phosphorylation of kinases indicated.

Cytochemical staining of TLR2 or NF-κB. Transfected HEK293 cell clones, as well as primary murine or human macrophages, the last isolated as $CD14^+$ peripheral blood leukocytes and cultured in 20% of autologous serum[44], were grown on slides. Cells were washed with PBS, permeabilized, and incubated with 50 μg/ml TLR2 specific mAb and/or anti NF-κB/p65 (polyclonal rabbit, Santa Cruz)[40]. Specific secondary αIgG antibodies were applied. Cell membranes were stained with labeled concanavalin A (Molecular Probes).

Inhibition of TLR2 dependent cell activation *in vitro* and *in vivo*. Transiently transfected HEK293 cells, murine RAW264.7, as well as primary macrophages were used[40]. 50 μg/ml of antibodies were applied 30 min prior to challenge with 100 ng/ml of LPS, IL-1β, P_3CSK_4, or 1 x 10^6 cfu/ml of h. i. *B. subtilis*. HEK293 cells were cotransfected with reporter[45], human CD14, human or mTLR2, and MD2 (provided by Tularik, Drs. Golenbock and Heine, as well as Miyake, respectively) expression plasmids, and NF-κB dependent reportergene activity was assayed after 6 h of stimulation[40]. TNFα concentrations in supernatants of RAW264.7 and primary murine macrophages, as well as NF-κB translocation in human macrophages[44] were analyzed 24 h and 90 min after challenge, respectively. For carrying out challenge and antibody dose dependent NF-κB dependent EMSA, as well as p38, Erk1/2, and Akt phosphorylation specific immunoblot analysis (Cell signaling, Frankfurt, Germany), RAW264.7 macrophages were used. 1 x 10^6 cells were pretreated with antibodies as described above at various concentrations and stimulated for 90 min (EMSA) or 30 min (kinase phosphorylation analysis)[40]. For analysis of TLR2 inhibition *in vivo*, mice were injected i. p. with 1 mg of T2.5 or left untreated. 1 h later, 100 μg of P_3CSK_4 and 20 mg of D-galactosamine were injected i. p. Serum concentrations of TNFα, IL-8, IL-6, and IL-12p40 in five unchallenged control mice were 0.05 ng/ml, 0.43 ng/ml, not detectable, and 0.44 ng/ml, respectively.

Systemic shock induction. In an experimental sensitization dependent model[27], mice were injected intravenously with 1.25 μg of murine IFNγ. 20 min later, mice were injected i. p. with 1 mg of mAb as indicated. 50 min after IFNγ injection, 100 μg of synthetic P_3CSK_4 and 20 mg of D-galactosamine were injected i. p. as well. The experimental high dose shock model encompassed a single i. p. injection of h. i. *B. subtilis* suspension (corresponding to 5 x 10^{10} cfu) with prior (1 h) or subsequent (1 h, 2 h, or 3 h) i. p. injection of 1 mg mAb as indicated.

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Figure legends

Fig. 1 Application of mAb T2.5 for specific detection of TLR2. Results of flow-cytometry of HEK293 cells stably overexpressing Flag-tagged mTLR2 (*a*) or human TLR2 (*b*), as well as primary *TLR2*$^{-/-}$ (*c*) and wild-type murine macrophages (*d*) by staining with mAb T2.5 (bold line, unfilled area). Negative controls represent cells not stained with a primary, but incubated with a mouse IgG specific secondary antibody (filled areas). For positive controls, Flag- (*a* and *b*) and mTLR2 (*c* and *d*) specific polyclonal antisera were used (normal line, unfilled area). For immunoprecipitation with T2.5, lysates of HEK293 cells overexpressing murine or human TLR2, as well as of murine RAW264.7 macrophages were applied as indicated (*e*). TLR2 precipitates were visualized by application of Flag (HEK293) or mTLR2 (RAW264.7) specific polyclonal antisera. Flag specific (αFlag) and protein G beads in the absence of antibodies (pG), as well as vector transfected HEK293 cells were used as controls. The size of TLR2 was 97 kDa.

Fig. 2 Subcellular localization of TLR2 *in vitro*. MAb T2.5 was used for cytochemical detection of overexpressed murine and human TLR2 (*a*), as well as endogenous murine (*TLR2*$^{+/+}$, wild-type) or human TLR2 in primary macrophages (*b*). Vector transfected HEK293 cells, as well as *TLR2*$^{-/-}$ primary macrophages were analyzed as staining controls. Concanavalin A was used for staining of cytoplasmic membranes. The bars in the lower right corners of each field represent a distance of 20 μm (*a*) or 10 μm (*b*) on the slides analyzed.

Fig. 3 Inhibitory effect of mAb T2.5 on cell activation *in vitro*. NF-κB dependent luciferase activities in HEK293 cells overexpressing either murine (*a*) or human TLR2 (*b*), as well as TNFα concentrations in supernatants of RAW264.7 (*c*) or primary murine macrophages (*d*) challenged with inflammatory stimulants are shown (ND, not detectable). Cells were incubated either with T2.5 or conT2 only (empty bars), or additionally challenged with IL-1β (*a*, *b*, horizontally hatched bars), ultra pure LPS (*c*, *d*, bold upward hatched bars), P_3CSK_4 (filled bars), or h. i. *B. subtilis* (downward hatched bars, *a* to *d*). MAb and challenge (P_3CSK_4, LPS) dependent NF-κB / p65 nuclear

translocation in human macrophages (*e*) was analyzed by cytochemical staining (Unstim., unstimulated). NF-κB dependent electro mobility shift assay (EMSA) and phosphorylation of MAP kinases Erk1/2 (pErk1/2) and p38 (pP38), as well as Akt (pAkt) were analyzed by applying nuclear or total extracts, respectively, from RAW624.7 macrophages (*f*, *g*). Cells were preincubated with the amounts of mAb T2.5 or conT2 indicated (μg/ml) and subsequently challenged with P_3CSK_4 or LPS for 90 min (*f*, arrows indicate specific NF-κB-DNA complexes) or 30 min (*g*, P38, specific immunoblot as positive control). Untreated cells (control) were analyzed as controls.

Fig. 4 TLR2 expression *in vivo*. Flow-cytometry of splenocytes and peritoneal washout cells from wild-type and *TLR2*$^{-/-}$ mice *ex vivo* immediately upon isolation ($n = 5$, cells pooled for each sample). CD11b^{+} splenocytes from mice challenged with LPS for 24 h were analyzed for surface and intracellular TLR2 expression (*a*) by staining with T2.5 (bold line, *TLR2*$^{+/+}$; filled area, *TLR2*$^{-/-}$). For analysis of TLR2 regulation upon infection (*b*, *c*), mice were either left uninfected (-) or infected with Gram-positive *B. subtilis* and sacrificed after 24 h (+). Upon staining of CD11b, cells were stained with T2.5 (TLR2) either without (*b*) or upon permeabilization (*c*). Numbers in quadrants represent the proportion of single or double stained cells, respectively, as compared to the total number of viable cells analyzed (%).

Fig. 5 Inhibitory effect of mAb T2.5 on host activation by microbial challenge *in vivo*. Mice were pretreated i. p. with 1 mg mAb T2.5 (filled columns) or left untreated (unfilled columns). Mice were challenged after 1 h i. p. with P_3CSK_4 and D-galactosamine, as well as sacrificed 2 h or 4 h later ($n = 4$ for each group at each time point). Serum concentrations of TNFα (*a*), IL-8 (*b*), IL-6 (*c*), and IL-12p40 (*d*) were analyzed by ELISA ($^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$, student's *t* test for unconnected samples).

Fig. 6 Protective effect of mAb T2.5 on TLR2 dependent systemic challenge. IFNγ and D-galactosamine sensitized mice received either no mAb or 1 mg of mAbs T2.5 or conT.2 i. p. 30 min

prior to microbial challenge (*a*) with bacterial lipopeptide analogue (P_3CSK_4, ▲, no mAb, $n = 4$; O, mAb conT2, $n = 3$; ■, mAb T2.5, $n = 4$). Mice challenged with a high dose of h. i. *B. subtilis* were left untreated or treated with mAbs at different time points (*b*). Survival was monitored as indicated. Administration of TLR2 specific mAb T2.5 1 h prior to (-1 h), as well as 1 h (+1 h), 2 h (+2 h), and 3 h after (+3 h) microbial challenge (*b*, ▲, no mAb, $n = 8$; O, mAb conT2, -1 h, $n = 3$; ◆, mAb T2.5, -1 h, $n = 4$; □, mAb T2.5, +1 h, $n = 2$; x, mAb T2.5, +2 h, $n = 2$; ◇, mAb T2.5, +3 h, $n = 4$).

08-06/2003Pa

TLR2 antagonistic antibody and use thereof

Claims

1. An antagonist, which specifically inhibits or blocks the mammalian, preferably human, Toll-like receptor 2 (TLR2).

2. The antagonist of claim 1, which is an antibody, small molecule or an aptamer.

3. The antibody of claim 2, which is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a chimeric antibody, or a synthetic antibody.

4. The antibody of claim 3, which is directed against the extracellular domain of TLR2.

5. The use of an antagonist of one or more of claims 1-4 in the prevention and/or treatment of acute and/or chronic inflammatory processes induced by bacterial infection.

6. A screening method for identifying an antagonist capable of inhibiting or blocking TLR2, comprising the steps of:

   (a) generating or providing mammalian TLR2,

   (b) contacting said TLR2 with a candidate antagonist,

   (c) detecting the inhibition or blocking of said candidate antagonist by a suitable detection method,

   (d) selecting a candidate antagonist that has been tested positive in step (c),

   (e) optionally repeating steps (a) – (d) with a suitably modified form of the candidate antagonist of step (d).

Summary of the invention

An antagonist, which specifically inhibits or blocks the mammalian, preferably human, Toll-like receptor 2 (TLR2).

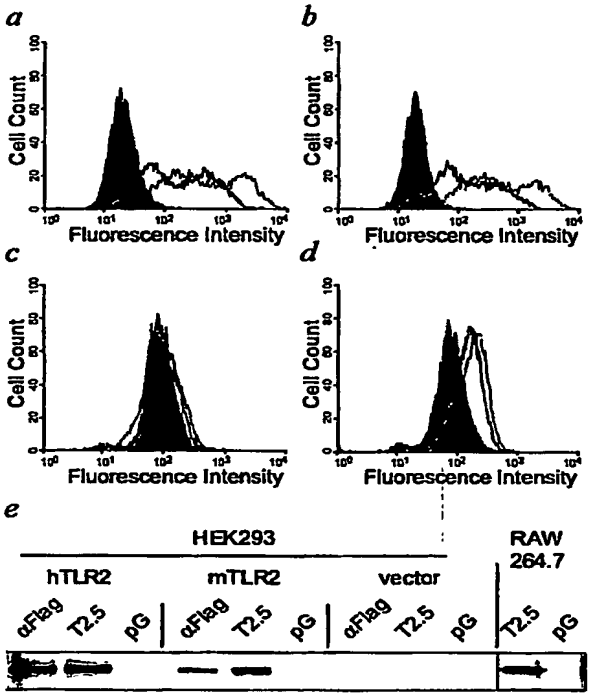


Fig. 1

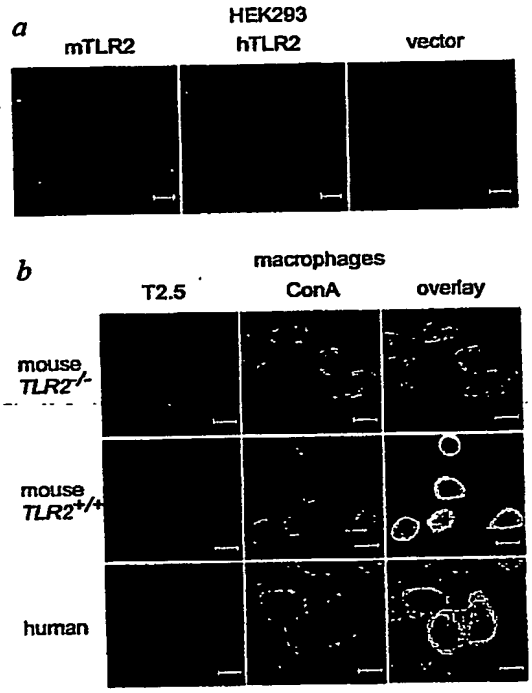
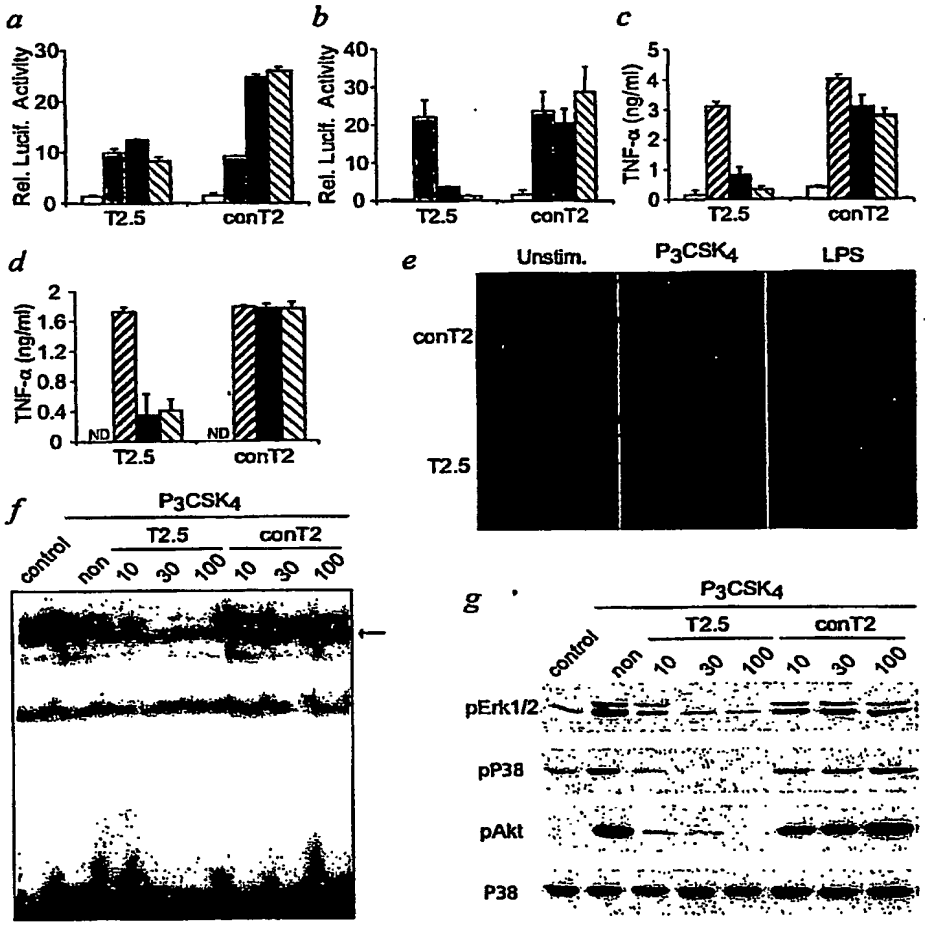

a
HEK293
mTLR2
hTLR2
vector
b
macrophages
T2.5
ConA
overlay
mouse TLR2-/-
mouse TLR2+/+
human

Fig. 2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Rel. Lucif. Activity
30
20
10
0
T2.5
conT2
b
Rel. Lucif. Activity
40
30
20
10
0
T2.5
conT2
c
TNF-α (ng/ml)
5
4
3
2
1
0
T2.5
conT2
d
TNF-α (ng/ml)
2
1.6
1.2
0.8
0.4
0
ND
ND
T2.5
conT2
e
Unstim.
P3CSK4
LPS
conT2
T2.5
f
P3CSK4
T2.5
conT2
control
non
10
30
100
10
30
100
g
P3CSK4
T2.5
conT2
control
non
10
30
100
10
30
100
pErk1/2
pP38
pAkt
P38

Fig. 3

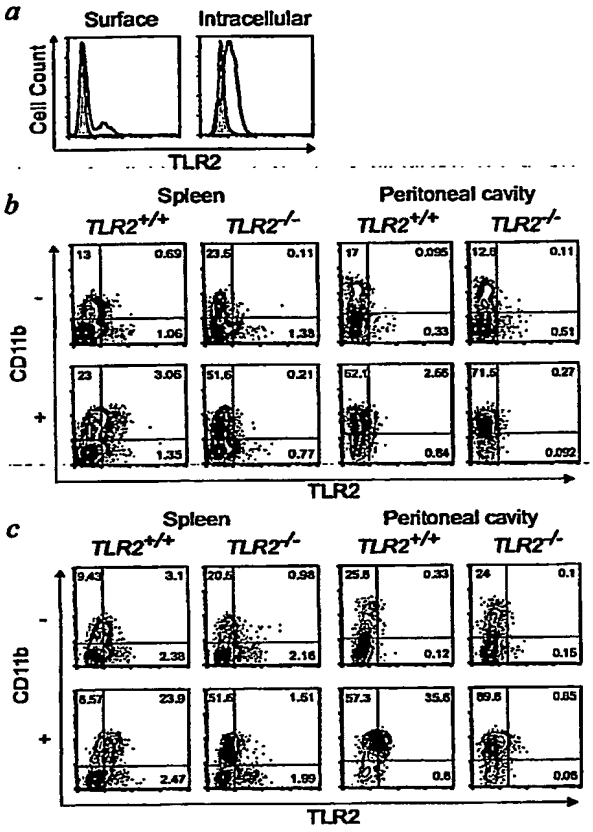
a
Surface
Intracellular
Cell Count
TLR2
b
Spleen
Peritoneal cavity
TLR2+/+
TLR2-/-
TLR2+/+
TLR2-/-
CD11b
TLR2
c
Spleen
Peritoneal cavity
TLR2+/+
TLR2-/-
TLR2+/+
TLR2-/-
CD11b
TLR2

Fig. 4

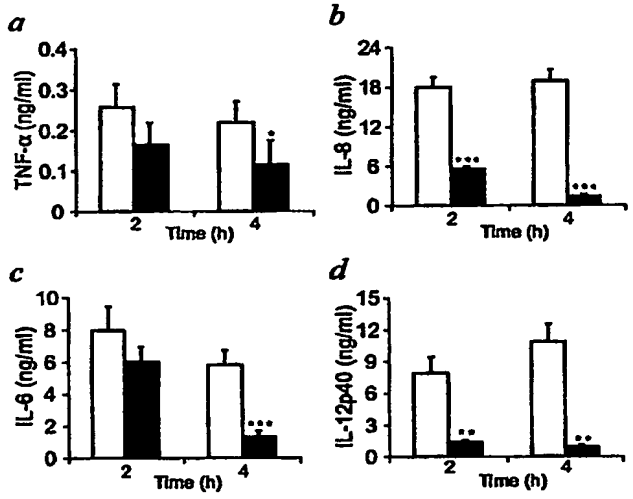
a
TNF-α (ng/ml)
0.4
0.3
0.2
0.1
0
2
4
Time (h)
*
b
IL-8 (ng/ml)
24
18
12
6
0
2
4
Time (h)
***
***
c
IL-6 (ng/ml)
10
8
6
4
2
0
2
4
Time (h)
***
d
IL-12p40 (ng/ml)
15
12
9
6
3
0
2
4
Time (h)
**
**

Fig. 5

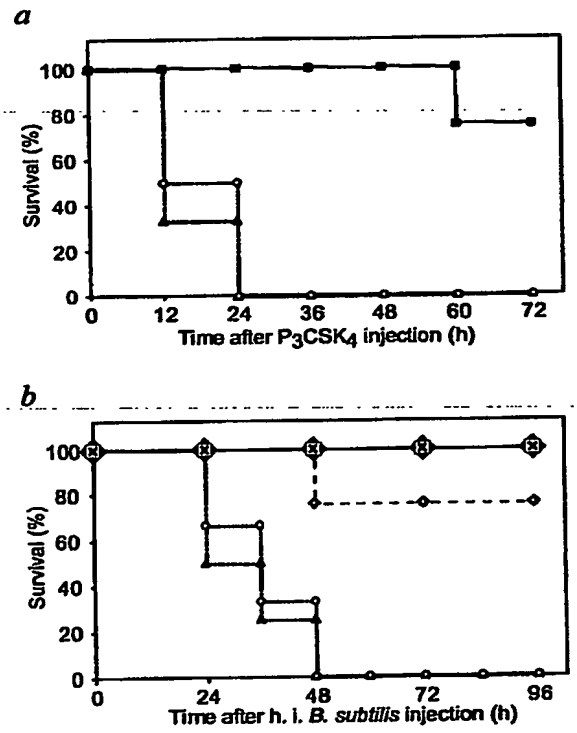
a
100
80
60
40
20
0
Survival (%)
0
12
24
36
48
60
72
Time after P3CSK4 injection (h)
b
100
80
60
40
20
0
Survival (%)
0
24
48
72
96
Time after h. i. B. subtilis injection (h)

Fig. 6

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